Protocol for the Isolation of RNA from Rodent Heart

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Supplies

Powder-free latex gloves
19 or 20 gauge x 1 to 1.5 inch needle
20 ml syringe
RNAlater™ (Supplier: Ambion, Austin TX)
Scissors
Forceps
70% alcohol prepared with RNase free water
Sterile gauze (2x2)
15 ml conical tube
2 ml cryovial (mouse heart)
5 ml cryovial (rat heart): The rat heart can be cubed and placed in multiple 2 ml cryovials.

Reminder: When collecting tissue for RNA, it is important to wear gloves and keep a clean, sterile environment. Work quickly to prevent degradation of the RNA.

Tissue Collection

Lightly anesthetize the animal with CO_2.
Pin down the front legs of the animal. Generously wet the underside fo the animal with 70% alcohol and wipe with sterile gauze. This will minimize the transfer of animal hair to the instruments and inside the body cavity.
Beginning 1 inch from the base of the sternum, open the animal up to the salivary glands. Remove the sternum.
Move the thoracic organs (lung and heart) out of the way. They can be pulled to the side or lifted with forceps. While the dorsal aorta is being clipped, gently begin flushing the heart with RNAlater™ by placing the needle, bevel side up, in the middle of the right ventricle. After all the RNAlater™ is expressed, quickly remove the heart, cut in half, leaving the apical aspect in tact. This will allow for the prosecuter to hold the heart with forceps and gently rinse in 5-10 mls of RNAlater™ to ensure blood is removed from the heart.
The heart can then be stored in cold RNAlater™ (5 mls – mouse/10 mls –rat) for up to one month at 4^{o}C before RNA isolation. Alternatively, the heart can be stored overnight at 4^{o}C in RNAlater™, removed from the solution the following day, placed in a cryovial and stored at –80^{o}C indefinitely. When ready to isolate RNA, remove the sample from the freezer and thaw at room temperature in the cryovial. Proceed immediately with the RNA isolation.

RNA Isolation

Qiagen Midi RNA Isolation Kit (Catalogue No. 75144) and Proteinase K (Cat. No 19131). This is their protocol (as found in Second Edition of their kit literature, June 2001, pp. 82-87) with minor modifications. In our hands, yields have varied from 0.5 to 1.0 µg of RNA/mg tissue.

Prepare Buffer RLT by adding 10µl/ml of ß-mercaptoethanol (~2 mls/heart). Add 4 volumes of ethanol (96-100%) to buffer RPE as indicated on the bottle. Turn on a water bath or other heating device to 55^{o}C to prepare for proteinase K digestion step.
Tare a medium sized weigh boat containing ~3-5mls of RNAlater™.
Working quickly, place the frozen or RNAlater™ preserved heart in the weigh boat and record weight. (Adult rodent heart weights range from 80 to 170 mg. If the heart weighs more than 150mg SLIT TISSUE INTO TWO POOLS AND PROCESS SEPARATELY.) Remove from the balance and with a new, clean razor blade chop the heart into ~ 1 - 2 mm square pieces.
Place in a 14 ml polypropylene round bottom tube (Falcon #2059) filled with 2 ml RLT buffer with ß-

mercaptoethanol. Homogenize immediately using a conventional rotor-stator homogenizer until the sample is uniformly homogenous (usually 45-60 s at maximum speed). Immediately proceed to step 5.
Add 4.0 ml of double-distilled water to the homogenate. Then add 65µl of Qiagen Proteinase K solution and mix thoroughly by pipetting.
Incubate at 55^{o}C for 20 min.
Centrifuge for 5 min at 3000-5000 x g.
Pipet the supernatant (approximately 6 ml) into a new 15 ml tube (not supplied).
Add 0.5 volumes (usually 3 ml) of ethanol (96-100%) to the cleared lysate. Mix well by pipetting. Do not centrifuge.
Pipet 3.0 ml of the sample, including any precipitate that may have formed, into an RNeasy midi column placed in a 15 ml centrifuge tube (supplied). Close the tube gently, and centrifuge for 5 min at 3000-5000 x g. Discard the flow through.
Repeat step 10, using another 3 ml of sample. Discard the flow through.
Repeat step 10 again, using the remainder of the sample (approximately 3 ml). Discard the flow through.
Add 4.0 ml Buffer RW1 to the RNeasy column. Close the centrifuge tube gently, and centrifuge for 5 min at 3000-5000 x g to wash. Discard the flow through.
Add 2.5 ml of Buffer RPE. Close the centrifuge tube gently, and centrifuge for 2 min at 3000-5000 x g to wash the column. Discard the flow through.
Add another 2.5 ml of Buffer RPE to the spin column. Close the centrifuge tube gently, and centrifuge for 5 min at 3000-5000 x g to dry the RNeasy membrane.
To elute, transfer the RNeasy column to a new 15 ml collection tube (supplied). Pipet 150µl of RNase-free water directly on the RNeasy silica-gel membrane. Close the tube gently. Let it stand 1 min, and then centrifuge for 3 min at 3000-5000 x g.
Repeat the elution step as described with a second volume of RNase-free water.
Quantitate and store the RNA as required. RNA should be snap frozen and stored at -80^{o}C or over liquid nitrogen in a LN_2 freezer. Keep on ice when pulled out to use.

For NIEHS in-house arrays: aliquots of 100µg should be stored at ~ 2µg/uµ in RNase-free water.

For Agilent commercial arrays: aliquots of 60 ug should be stored between 1.1 and 1.5µg/µl in RNase-free water.